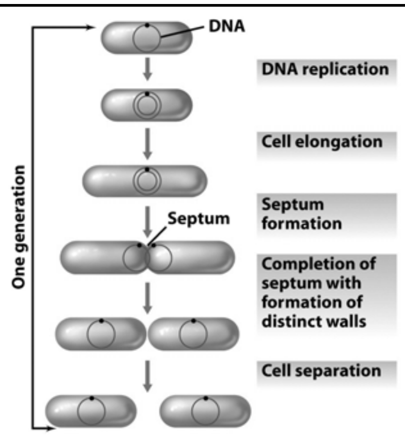
## The Process of Growth

- Metabolism required for growth, both anabolic and catabolic. ~2000 reactions!
- Usual Definition: **Increase in cell numbers**
  Other definitions possible – spores, UMC's, respiration, viable but nonculturable, morphology changes (life cycle)
- Divide via Binary Fission: 3 mechanisms involved!
  Cell Elongation – cell wall
  DNA Replication – rate limiting step
  Cell Division – septum formation



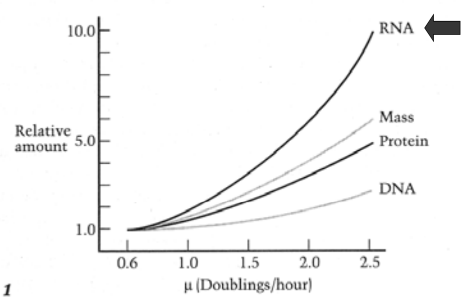




***Figure 1***

**Effect of nutrition-imposed growth rate on the composition of *Escherichia coli* B/r. All values are expressed in amounts per cell normalized to values at $\mu = 0.6$ (mass = $1.48 \times 10^{-13}$ g; protein = $1.00 \times 10^{-13}$ g; RNA = $2.0 \times 10^{-14}$ g; DNA = $6.3 \times 10^{-15}$ g). (Plotted from data in Bremer and Dennis, 1987.)**

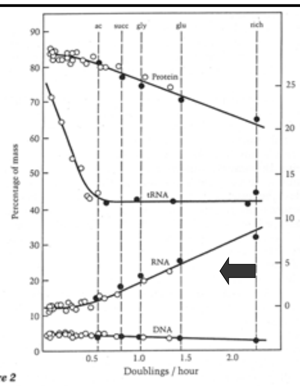
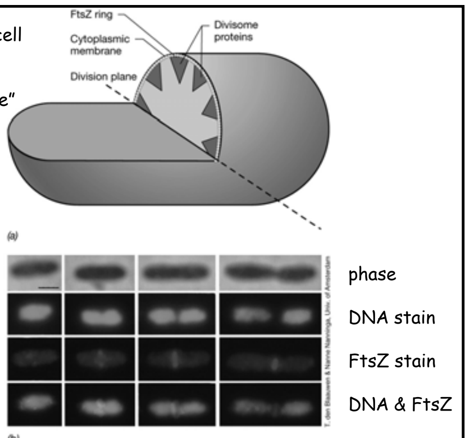



*Figure 2*

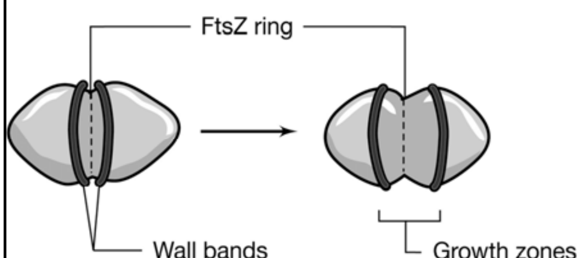
**Effect of growth rate on the cellular proportions of protein, RNA, and DNA. Filled circles refer to results from cultures undergoing balanced growth in batch culture in various media; open circles are from cultures growing in a glucose-limited chemostat. (From Jacobsen, 1974.)**

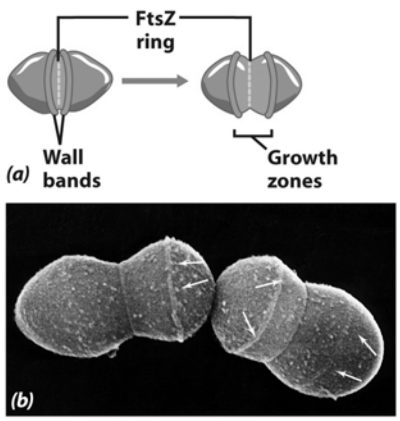
FtsZ ring & cell division

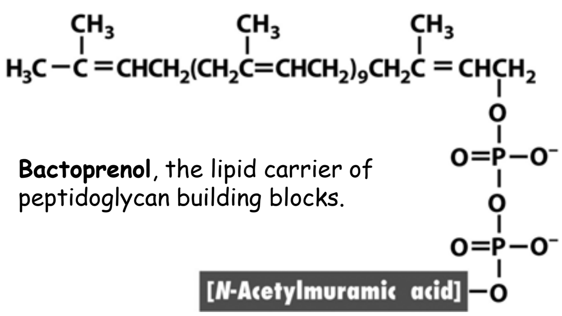
The "divisome"



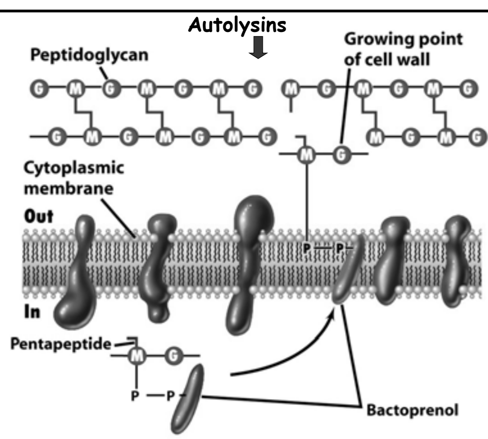

**FtsZ similar to Tubulin**
**MreB similar to Actin**



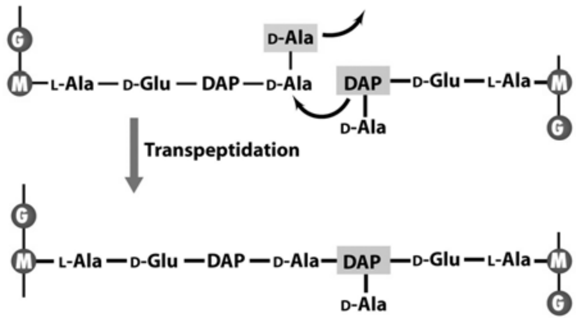



**Bactoprenol**, the lipid carrier of peptidoglycan building blocks.



**Penicillin** blocks this reaction



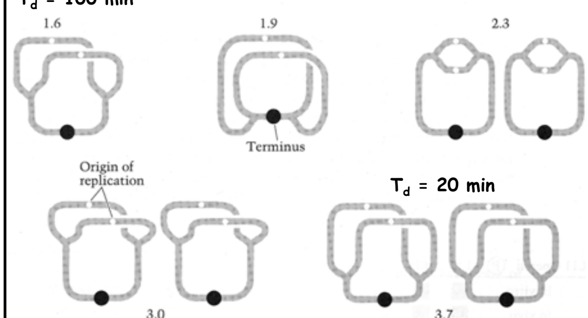




*Figure 10*

**Chromosome structure and equivalent DNA content of the average cell in culture of *E. coli* B/r growing at various rates. The numbers represent genome equivalents. (From Bremer and Dennis, 1987.)**

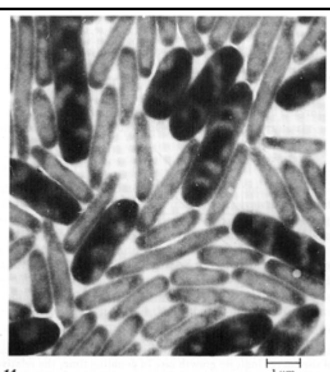



*Figure 14*

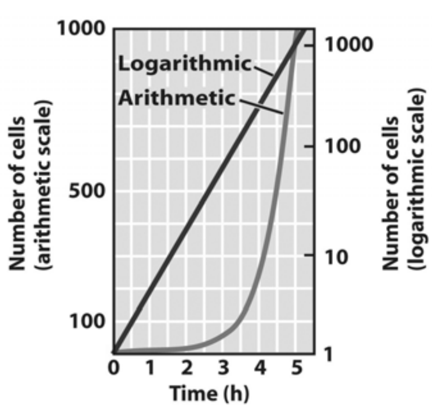
**Electron micrograph of a mixture of cells of *E. coli* B/r grown at different rates. The large cells grew with a doubling time of 22 minutes, the small ones with a doubling time of 72 minutes. (From Nanninga and Woldringh, 1985.)**

## The Process of Growth

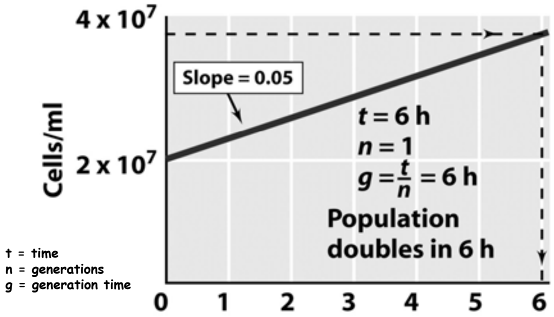
- Growth Rate: Time it takes to reproduce
  **$t_{\frac{1}{2}}$ = ln2/μ = 0.693/μ = g**
- Phases of Growth in Batch culture
  Lag, Log, Stationary, Death
- Measurement of Growth
  Total cell counts
  Viable cell counts
  Turbidity

**The growth rate of a microbial culture**

| Time (h) | Total number of cells | Time (h) | Total number of cells |
|---|---|---|---|
| 0 | 1 | 4 | 256 ($2^8$) |
| 0.5 | 2 | 4.5 | 512 ($2^9$) |
| 1 | 4 | 5 | 1,024 ($2^{10}$) |
| 1.5 | 8 | 5.5 | 2,048 ($2^{11}$) |
| 2 | 16 | 6 | 4,096 ($2^{12}$) |
| 2.5 | 32 | . | . |
| 3 | 64 | . | . |
| 3.5 | 128 | 10 | 1,048,576 ($2^{19}$) |



How to estimate the generation times of an exponential microbial culture using semi-log plots.



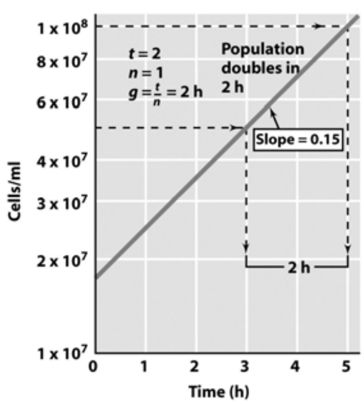



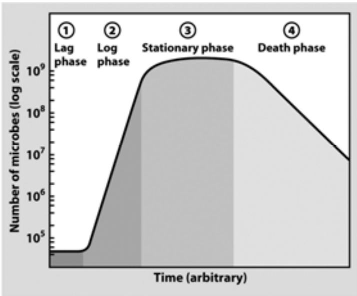

**Table 6.1** Approximate generation times for several organisms growing in media optimal for growth

| Species | Generation Time |
|---|---|
| *Escherichia coli* | 20 min |
| *Bacillus subtilis* | 28 min |
| *Staphylococcus aureus* | 30 min |
| *Pseudomonas aeruginosa* | 35 min |
| *Thermus aquaticus* | 50 min |
| *Thermoproteus tenax* | 1 hr 40 min |
| *Rhodobacter sphaeroides* | 2 hr 20 min |
| *Sulfolobus acidocaldarius* | 4 hr |
| *Thermoleophilum album* | 6 hr |
| *Thermofilum pendens* | 10 hr |
| *Mycobacterium tuberculosis* | 13 hr 20 min |

## The Growth Cycle

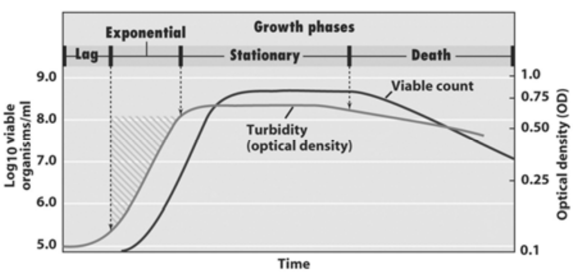
- Lag phase
  - Cells synthesizing materials, not dividing
- Log phase = exponential growth
  - 1 → 2 → 4 → 8 → 16 …
    - 10 doublings increases density by ~1000
    - $\log_{10}(N)$ increases linearly
- Stationary phase
  - Cells no longer growing
- Death phase

## The Growth Cycle



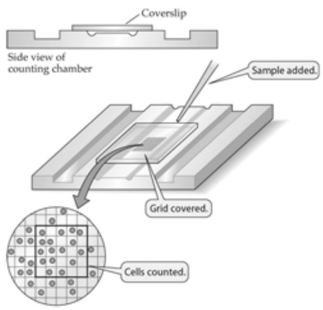

Log scale necessary to show wide range of concentrations

Cryptic Growth

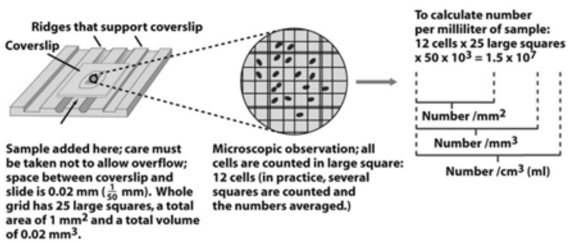



Typical growth curve for a bacterial population

## Total Cell counts using the Petroff-Hausser Counter

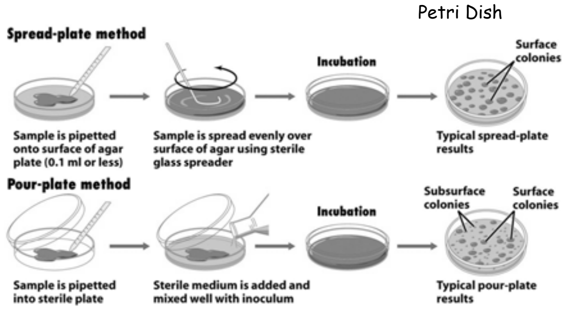



## Total Cell counts using the Petroff-Hausser Counter

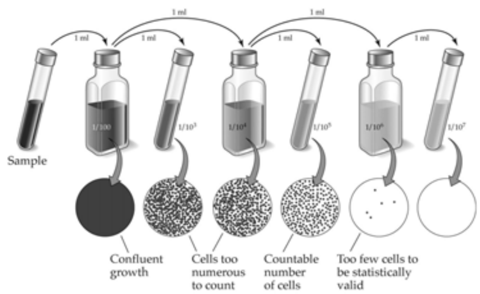



## Viable cell count methods

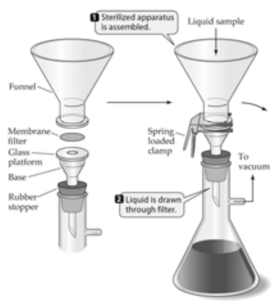
30-300 on standard Petri Dish



Counting the number of viable cells by serial dilution and plate count

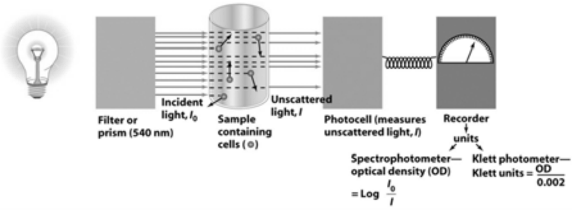



(Part 1) Concentration of cells by membrane filtration

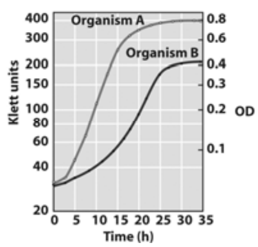


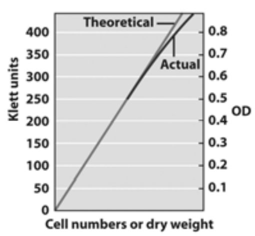

(Part 2) Concentration of cells by membrane filtration

Turbidity measurements of microbial growth




Turbidity measurements of microbial growth



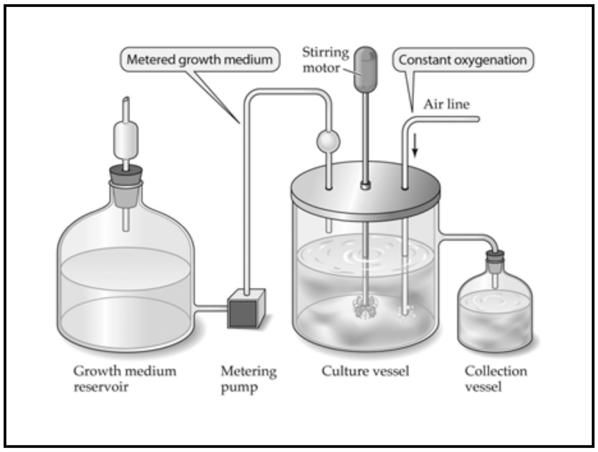



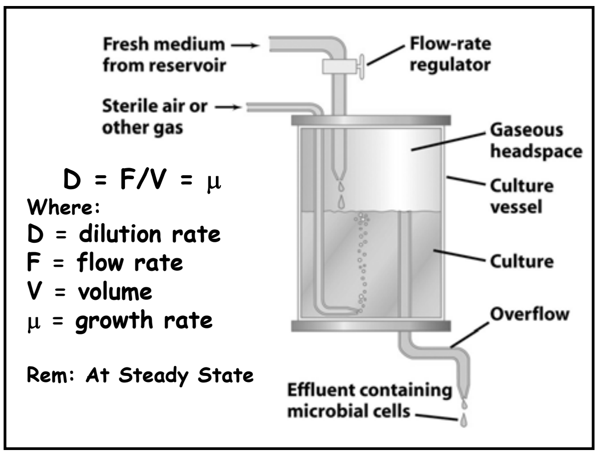

## The Process of Growth

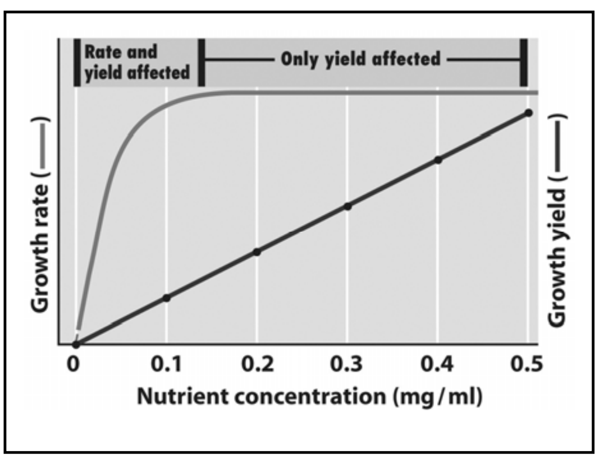
- Continuous Culture: The wonders of the **chemostat**
  - Steady State
  - Reproducible Physiology
  - Fine control

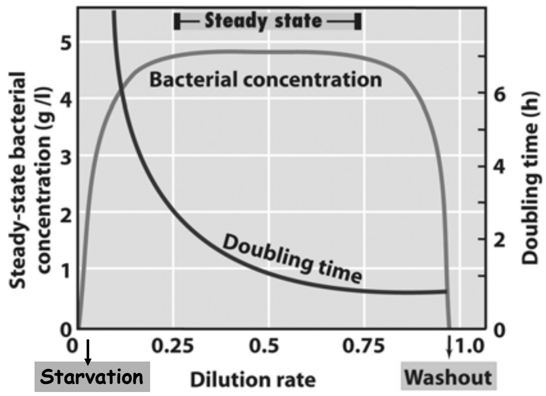
  Key parameters: Ks, μmax, Yield

  Closed systems vs. Open systems vs. Nature!


Metered growth medium
Stirring motor
Constant oxygenation
Air line
Growth medium reservoir
Metering pump
Culture vessel
Collection vessel


Fresh medium from reservoir
Flow-rate regulator
Sterile air or other gas
Gaseous headspace
Culture vessel
Culture
Overflow
Effluent containing microbial cells
D = F/V = μ
Where:
D = dilution rate
F = flow rate
V = volume
μ = growth rate
Rem: At Steady State


Rate and yield affected
Only yield affected
Growth rate (——)
Growth yield (——)
0
0.1
0.2
0.3
0.4
0.5
Nutrient concentration (mg/ml)



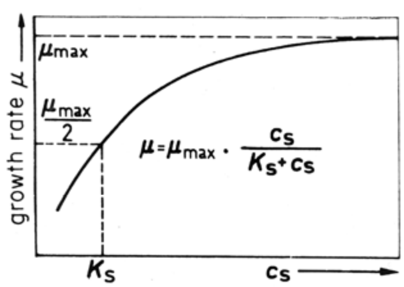




Fig. 6.10 *Dependence of growth rate $\mu$ on the substrate concentration* $c_s$.

Steady-state relationship between substrate concentration and output of bacterial mass

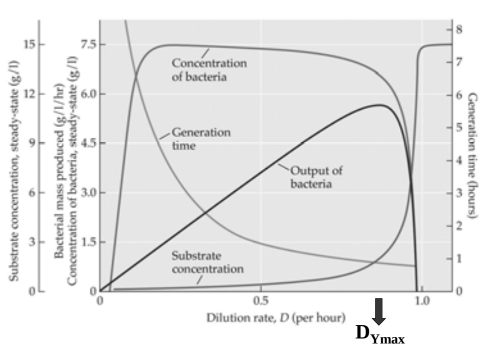


**Table 6.2** Growth yields of anaerobic bacteria utilizing glucose as the energy source

| | Mol ATP/Mol Glucose | $y_{max}$ (g of cell/mol Glucose) | $y_{ATP}$ (g of cell/mol ATP) |
|---|---|---|---|
| *Lactobacillus delbrueckii*[a] | 2 | 21 | 10.5 |
| *Enterococcus faecalis*[a] | 2 | 20 | 10 |
| *Zymomonas mobilis*[b] | 1 | 9 | 9 |

[a]Homolactic fermentation, Embden–Meyerhof pathway (see Chapter 10).
[b]Alcoholic fermentation, Entner–Doudoroff pathway (see Chapter 10).